

Argyrophilic Nucleolar Organizer Regions: Their Value and Correlation With Clinical Prognostic Factors in Breast Carcinoma

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Background and Objectives: Argyrophilic nucleolar organizer regions (AgNORs) have been recently identified as a marker of proliferative index in various tumors. These were evaluated in 46 patients with primary breast carcinoma and were correlated with clinical prognostic parameters of breast cancer. Ten patients with benign breast tumors served as controls in the study.

Methods: AgNORs were stained in paraffin sections of the tissues using Ploton's silver technique. For each specimen, the number of AgNORs within the nuclei of 100 tumor cells were calculated. The average number of AgNORs per nucleus was calculated and the results expressed as mean \pm S.D.

Results: AgNOR count was significantly higher in breast carcinoma (6.61 ± 1.75) than in benign breast tumors (1.88 ± 0.19). Further, the AgNOR count in breast carcinoma showed a statistically significant increase in correlation with the increase in the size of the tumor, stage of the cancer, number of metastatic lymph nodes, and tumor recurrence at various sites. However, the differences in AgNOR count at different lymph node levels and histologic grading were not statistically significant.

Conclusions: These results indicate that breast tumors with a higher AgNOR count, even at the initial stage, have a poor prognosis and require aggressive treatment for better control of the disease. Further, it is suggested that the patients with a benign tumor and more than three AgNORs per nucleus need careful surveillance.

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KEY WORDS: AgNORs; prognosis; breast cancer

INTRODUCTION

Breast carcinoma is the second most common malignancy in Indian females. The defined prognostic variables, such as clinical stage, number of lymph nodes, growth factor receptors, estrogen/progesterone receptor, and histological grading, play an important role in the favorable outcome of the cancer. In addition to conventional pathological parameters, several new methods provide information concerning the malignant potential of tumors [1,2]. In view of the fact that aneuploid tumors

with high proliferative index have an increased rate of recurrence and carry a poor prognosis [3], the measurement of DNA content of tumor cells by flow cytometry and immunohistochemistry is widely used as a diagnostic and prognostic tool [4,5]. Nucleolar organizer regions

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TABLE I. Argyrophilic Nucleolar Organizer Regions Count in Benign and Malignant Breast Tumors*

Group of samples	AgNOR count per cell (mean \pm SD)
Benign (n = 10)	1.88 \pm 0.19
Malignant (n = 46)	6.65 \pm 1.75

*Benign vs. malignant, $t = 8.453$, $P < 0.001$.

(NORs) are loops of ribosomal DNA (rDNA) located on the short arms of the acrocentric chromosomes 13, 14, 15, 21, and 22 [6,7] and are associated with rRNA transcription and regulates protein synthesis. These are identified by silver nitrate stain in sections of paraffin-embedded tissues and are referred to as the silver nucleolar organizer regions (AgNORs). The AgNOR counts also have been associated directly with cellular and nuclear activity and are used as diagnostic and prognostic indicators in various growing tumors [8–10]. The numerical value of AgNORs, their morphology, and distribution are considered as determinants of malignant transformation. Their value in breast cancer also has been suggested, but the results reported are controversial [11,12].

The present study was undertaken to evaluate the usefulness of AgNOR counts in breast cancer and to correlate them with clinical prognostic variables.

MATERIALS AND METHODS

Forty-six patients with breast carcinoma and 10 patients with benign breast tumors were evaluated between January 1991 and January 1993. Their clinicopathological data, including size, stage, histologic grade, lymphnode metastasis, and level of nodes, were examined and correlated with mean AgNOR count. The resected tissues were fixed in 10% formal saline, dehydrated in graded series of ethyl alcohol, and embedded in paraffin. Sections of 2–3 μ m were cut and stained with hematoxylin and eosin (H&E) for histopathological examination of the tissues.

The silver staining technique of Ploton et al. [10] was used to stain AgNORs. The stained specimens were examined under oil immersion lens at a magnification of $\times 1,000$. For each analysis, 100 randomly selected tumor cells in each tissue specimen were examined and the number of AgNOR per nucleus was counted independently by two observers. The results were expressed as mean \pm SD throughout.

Differences were analyzed by Student's t -test. P values of <0.05 were considered significant.

RESULTS

Most of the patients (76.09%) were between 40–60 years old. Of these, 69.56% had stage III disease at the time of presentation. Infiltrating duct carcinoma was the most common histological type. Table I shows a high

TABLE II. Correlation of Argyrophilic Nucleolar Organizer Regions (AgNOR) Count With Size of the Breast Lump

T stage*	Size of breast lump (cm)	AgNOR count/cell mean (\pm SD)	T value	P value
T ₁	<2	3.21 \pm 1.42	2.683	<0.02
T ₂	2–5	4.95 \pm 1.54	6.028	<0.001
T ₃	5–10	6.24 \pm 1.38	9.561	<0.001
T ₄	>10	7.85 \pm 1.25	14.106	<0.001
Control		1.88 \pm 0.19		

*T₁ vs. T₂, $t = 2.398$ $P < 0.05$; T₂ vs. T₃, $t = 2.459$ $P < 0.02$; T₃ vs. T₄, $t = 2.808$ $P < 0.01$.

AgNOR count per cell in breast carcinoma (6.65 \pm 1.75), in contrast to the counts in benign breast tumors (1.88 \pm 0.19) ($P < 0.001$). Table II shows an increase in AgNOR count from 3.21 \pm 1.42 to 7.85 \pm 1.25 with the increase in tumor size from 2 cm to 10 cm, respectively ($P < 0.001$). There was an increase in AgNOR count from stage I to stage IV breast carcinoma of 3.0 \pm 1.1 and 7.75 \pm 1.38, respectively ($P < 0.001$) (Table III). Table IV shows AgNOR count 3.41 \pm 0.48 and 6.80 \pm 1.29 in breast carcinoma tissues of patients with the involvement of <4 lymph nodes and >4 lymph nodes, respectively ($P < 0.001$).

The AgNOR count in patients with breast carcinoma with the involvement of lymph nodes at levels I, II, and III are summarized in Table V. The difference in the counts at different levels of lymph nodes is not statistically significant ($P > 0.05$). Patients with high AgNOR count at the time of initial diagnosis of carcinoma developed recurrences and distant metastases during follow-up treatment (Table VI) and thus need careful surveillance.

DISCUSSION

Breast carcinoma behave differently in different individuals and the behavioral changes in the tumor determine the final outcome of the disease. Indicators of tumor behavior are the proliferative index, DNA content, or ploidy. Different methods of assessment of the indicators have been described [13]. The number of AgNORs per nucleus has recently been introduced as a good marker of the proliferative activity of various tumors [14]. Its count showed a consistent rise from benign to malignant transformation in tumors. The AgNOR in benign tumors is reported to be 1–2 per nucleus and any number >2 is suggestive of increased DNA content or aneuploidy [15].

The present study indicates that AgNOR count >3 is strongly suggestive of malignancy. This knowledge may be utilized to study patients who are at high risk and to determine which are borderline lesions. Thus patients with AgNOR counts >3 , although having histologically benign tumors, require closer surveillance.

The present study shows that in breast carcinoma,

TABLE III. Correlation of Argyrophilic Nucleolar Organizer Regions (AgNORs) Count With the Stage of the Breast Cancer*

Stage of disease	AgNOR count per cell (mean \pm SD)	<i>t</i> value	<i>P</i> value
I	3.0 \pm 1.1	—	—
II	4.71 \pm 1.42	5.903	<0.001
III	6.01 \pm 1.58	8.049	<0.001
IV	7.75 \pm 1.38	12.168	<0.001
Control	1.88 \pm 0.19		

*Comparison between groups: Stage II vs. Stage III, $t = 0.173$ $P < 0.05$; Stage III vs. Stage IV, $t = 2.045$, $P < 0.05$.

TABLE IV. Correlation of Argyrophilic Nucleolar Organizer Regions (AgNORs) Count With Number of Lymph Nodes Involved in Patients With Breast Cancer*

Number of metastatic lymph nodes	AgNOR count per cell (mean \pm SD)	<i>t</i> value	<i>P</i> value
<4	3.41 \pm 0.048	9.099	<0.001
>4	6.80 \pm 1.29	11.738	<0.001
Control	1.88 \pm 0.19		

*Comparison within groups: <4 vs. >4 nodes, $t = 9.024$, $P < 0.001$.

TABLE V. Correlation of Argyrophilic Nucleolar Organizer Regions (AgNORs) Count With Levels of Lymph Nodes Involvement in Breast Cancer

Levels of lymph nodes*	AgNORs count per cell (mean \pm SD)	<i>t</i> value	<i>P</i> value
I	6.41 \pm 2.10	6.690	<0.001
II	6.51 \pm 2.29	6.174	<0.001
III	6.57 \pm 3.09	4.540	<0.001

*Level I vs. Level II, $t = 0.172$ $P > 0.05$; Level I vs. Level III, $t = 0.191$ $P > 0.05$; Level II vs. Level III, $t = 0.053$ $P > 0.05$.

TABLE VI. Correlation of Argyrophilic Nucleolar Organizer Regions (AgNORs) Count at Initial Presentation With Metastatic Pattern of Breast Cancer During Follow-Up

Metastatic sites	Initial AgNOR count per cell (mean \pm SD)	<i>t</i> value	<i>P</i> value
Local recurrence	7.38 \pm 1.29	12.157	<0.001
Regional recurrence	6.51 \pm 1.18	10.657	<0.001
Metastatic liver	7.75 \pm 1.58	10.556	<0.001
Pleural effusion	6.43 \pm 1.88	6.815	<0.001

AgNOR count rises with increase in tumor size. This corroborates the findings of Ohri et al. [11], but is not in conformity with the observations of Raymond et al. [16]. AgNOR count as a prognostic parameter has been reported in hematological malignancy and various solid tumors, including breast carcinoma. The results reported in the literature are conflicting with regard to the stage of the disease and AgNOR count. Since increased AgNOR

count reflects cellular proliferation, it is likely that it could be a prognostic parameter for an aggressive tumor.

The present study does not show any correlation between AgNOR counts and histological grade of malignancy. This observation corroborates the previous reports [12,16] and is in contradiction to the observations of Eskelinen et al. [17]. The lack of unanimity in various studies could be because of the variation in sample size.

The comparable data on the level of lymph node and AgNOR counts are not available. However, they were statistically insignificant in the present study ($P > 0.05$). A significant increase in the count ($P < 0.001$) was found in patients with >4 metastatic lymph nodes, or with extensive systemic metastases. These observations are again accountable for increased proliferative activity of the tumor cells [9]. The study indicates that patients with an initial higher AgNOR count are prone to develop local and systemic failures and had a poor prognosis. Hence these patients may require aggressive combined modality of treatment for a favorable outcome.

Although the precise nature and function of the argyrophilic nucleolar regions remain undetermined, the present study suggests that AgNOR counts in breast carcinoma may be of value in predicting high risk patients and also may be an indicator of aggressiveness.

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